ORIGINAL ARTICLE

Diagnosis and prevalence of diabetic polyneuropathy: a cross-sectional study of Danish patients with type 2 diabetes

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Background and purpose: Diabetic polyneuropathy (DPN) is a common complication of diabetes. Using the Toronto criteria for diabetic polyneuropathy and the grading system for neuropathic pain, the performance of neuropathy scales and questionnaires were assessed by comparing them to a clinical gold standard diagnosis of DPN and painful DPN in a cohort of patients with recently diagnosed type 2 diabetes.

Methods: A questionnaire on neuropathy and pain was sent to a cohort of 5514 Danish type 2 diabetes patients. A sample of 389 patients underwent a detailed clinical examination and completed neuropathy questionnaires and scales.

Results: Of the 389 patients with a median diabetes duration of 5.9 years, 126 had definite DPN (including 53 with painful DPN), 88 had probable DPN and 53 had possible DPN. There were 49 patients with other causes of polyneuropathy, neuropathy symptoms or pain, 10 with subclinical DPN and 63 without DPN. The sensitivity of the Michigan Neuropathy Screening Instrument questionnaire to detect DPN was 25.7% and the specificity 84.6%. The sensitivity of the Toronto Clinical Neuropathy Scoring System, including questionnaire and clinical examination, was 62.9% and the specificity was 74.6%.

Conclusions: Diabetic polyneuropathy affects approximately one in five Danish patients with recently diagnosed type 2 diabetes but neuropathic pain is not as common as previously reported. Neuropathy scales with clinical examination perform better compared with questionnaires alone, but better scales are needed for future epidemiological studies.

Introduction

Diabetic polyneuropathy (DPN) is a length-dependent symmetrical sensorimotor polyneuropathy and is one of the most common and troublesome complications of diabetes [1]. The reported prevalence of DPN ranges from 13% to 55% [2–4], and 25%–50% of these patients have neuropathic pain [3,5]. The large variation in the prevalence may be explained by differences in study populations (e.g. diabetes duration and type) and different criteria for polyneuropathy and neuropathic pain.

Correct and early identification of patients with DPN is essential for preventive purposes. Finding a

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screening instrument that is simple and easy to administer with a high positive and negative predictive value is of importance. However, the identification of DPN and painful DPN is not straightforward [6]. The current gold standard for DPN is the hierarchical grading system by the Toronto Diabetic Neuropathy Expert Group and for neuropathic pain the NeuPSIG (the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain) grading system. These require a medical history, neurological examination, and neurophysiological or neuropathological testing [7,8].

Clinical scores based on recorded symptoms and signs are expected to have higher diagnostic accuracy than symptoms alone [9], but questionnaires are useful in large epidemiological studies [10]. The Michigan Neuropathy Screening Instrument questionnaire part (MNSIq) has been used to identify DPN and is validated for DPN in patients with type 1 diabetes [2,11,12]. Similarly, the Douleur Neuropathique 4 questionnaire (DN4q) is a screening tool used to identify painful DPN [10,13].

Only a few large studies, which included a detailed examination done by experienced neurologists employing clinical, neurophysiological and neuropathological consensus criteria, have assessed the prevalence of DPN and often in unclear study populations [3,14]. In this study, a detailed clinical examination in a sample of a large well-characterized nationwide cohort of patients with recently diagnosed type 2 diabetes was performed using the Toronto classification system and the NeuPSIG grading system as gold standards for DPN and neuropathic pain, respectively. The aim was to determine the prevalence of DPN in newly diagnosed type 2 diabetes patients and to evaluate critically the diagnostic performance of questionnaires and neuropathy scales.

Research design and methods

This was a cross-sectional population-based study of patients with recently diagnosed type 2 diabetes in Denmark, conducted at two study sites, Aarhus and Odense. Patients were recruited from a questionnaire survey study conducted in 2016 amongst 5514 patients from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort. In the questionnaire survey study, all patients were screened for symptoms of DPN and painful DPN using the MNSIq and DN4q and the prevalence of possible DPN and painful DPN was calculated [15].

In order to limit patient transportation, all patients living close to the two study sites were invited, where the only requirement for invitation was a valid answer on the questionnaire survey (random sample). To ensure a larger number of patients with DPN, patients with symptoms of neuropathy based on the questionnaire survey study (MNSIq ≥ 4 and/or DN4q ≥ 3) from the rest of Denmark, except the Capital region and the Eastern part of Zealand (selected sample), were invited. The Danish healthcare system is tax paid and offers free and equal access to all citizens; therefore, regional differences in healthcare access and treatment were not expected. The patient inclusion is illustrated in Fig. 1. One reminder was sent to those who did not reply.

Exclusion criteria were cognitive impairment, language difficulties and pregnancy. Patients were included from October 2016 to the end of October 2018. In addition, 97 subjects without diabetes of similar age and sex were included during the same period, recruited from within the patients' social or work circle and by flyers. Baseline characteristics and inclusion/exclusion criteria for this group are provided in Table S1.

The two clinical investigators at each center were trained neurologists (S.S.G. and M.I.). They interviewed all patients, focusing on the presence of symptoms of polyneuropathy including duration, localization and type of symptoms, medication use and comorbidities. On the same day, all patients filled in a questionnaire, including questions on lifestyle habits (smoking, alcohol consumption and physical activity), psychological functions and the MNSIq and DN4q.

Height and weight were measured, and waist circumference (average of two measurements between the lower ribcage and iliac crest after expiration in standing position) was measured. Blood samples for glycated hemoglobin (HbA_{1c}), non-fasting blood sugar levels, cholesterol and triglycerides were taken.

A detailed neurological examination of lower extremities was conducted, including a standard sensory examination and mapping for the following modalities: light brush stroking (Somedic AB, Hörby, Sweden); pinprick (Semmes–Weinstein monofilament no. 5.88, bending force 75.9 g/745 mN; Stoelting, Wood Dale, IL, USA); and cold (20°C) and warm (40°C) thermal rolls (Somedic AB, Hörby, Sweeden). The upper thigh or chest was used as the control area. Knee and ankle reflexes were assessed with a Tromner reflex hammer (US Neurologicals LLC, USA) and muscle strength was assessed in accordance with the Medical Research Council scale. Vibration sense was determined with both a 128 Hz tuning fork and a biothesiometer (Biomedical Instruments, OH, USA) on the dorsum of the great toe. Lastly, the clinical examination parts of the MNSI [16], DN4 [17], the Toronto

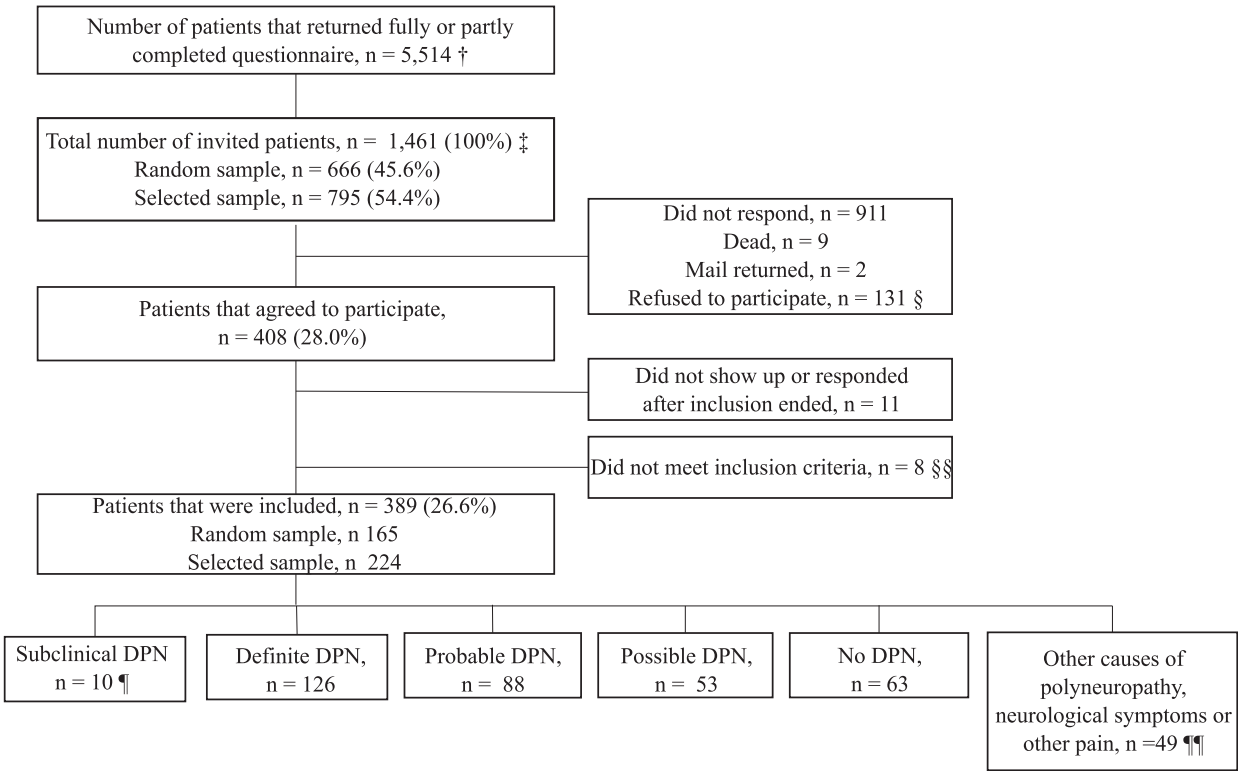

Figure 1 Flowchart of patient inclusion and patient groups/diagnosis according to the Toronto criteria [8] and the NeuPSIG grading of neuropathic pain [7]. †Questionnaires were sent to 6726 patients enrolled in the DD2 cohort by February 2016. ‡Reminders were sent to 72.7% of those not responding to the first invitation (because of study completion, not all received a reminder) and 7.1% confirmed to participate. §Of the 131 that refused to participate, $n = 71$ gave reasons: had no time, energy or were not interested ($n = 54$), other acute or chronic diseases than diabetes ($n = 13$), living abroad ($n = 2$) and geographical distance ($n = 2$). §§Reasons for exclusion: psychiatric disease ($n = 1$), dementia and other cognitive problems ($n = 5$) and language difficulties ($n = 2$). ¶Subclinical DPN: patients with no symptoms or signs of DPN and abnormal NCS ($n = 5$) or IENFD ($n = 5$). ¶¶Other causes of polyneuropathy: chemotherapy- or alcohol-induced polyneuropathy, chronic inflammatory demyelinating polyneuropathy, sarcoidosis, vitamin B12 deficiency, infection with human immunodeficiency virus and psoriasis arthritis. Other diseases causing pain or neurological symptoms: spinal stenosis, arthritis (osteo-, psoriasis, borrelia-), herniated disc, multiple sclerosis, fibromyalgia, peripheral arterial disease, stroke, transversal myelitis, sequela from trauma and operations (in the back and feet), pes planus transverse, progressive supranuclear palsy and restless leg syndrome. DD2, Danish Centre for Strategic Research in Type 2 Diabetes; DPN, diabetic polyneuropathy; IENFD, intraepidermal nerve fiber density; NCS, nerve conduction studies. In the group of 389 patients, skin biopsies for IENFD were not taken for 55/389 and NCS were not conducted on 9/389. Three patients had neither IENFD nor NCS results and could therefore only be classified as maximum probable DPN.

Clinical Neuropathy Scoring System (TCNS) [18,19] and the Utah Early Neuropathy Scale (UENS) [20] were performed. Agreement between the primary investigators (S.S.G. and M.I.) was ensured by making detailed descriptions of all procedures, joint training, by regularly examining patients together during the inclusion period, and by comparing results and conclusions.

A single 3 mm punch biopsy was taken 10 cm above the right lateral malleolus for determination of the intraepidermal nerve fiber density (IENFD) with subcutaneous anesthesia using 10 mg/ml lidocaine if needed. The fixation, cryoprotection, staining and analysis methods are described in detail elsewhere [21]. IENFD was considered abnormal if it was lower than the fifth percentile compared to age- and sex-matched healthy controls, as previously published [22]. Amongst the included controls without diabetes, two (2%) had abnormal IENFD (Table S1).

Conventional nerve conduction studies (NCS) of sural nerves were carried out bilaterally, the median, peroneal and tibial nerves unilaterally [23]. The ulnar nerve was examined on the same side as the median nerve if the median nerve was found to be abnormal. The results were compared to laboratory controls using z-scores. Polyneuropathy was defined as two or more nerves with one or more abnormal measure, including at least one abnormal sural nerve [23].

Quantitative sensory testing (QST) was performed using a reduced version of the standardized protocol of the German Research Network for Neuropathic Pain [24]. Perception thresholds for warmth, cold, vibration, pinprick and pain were examined as well as dynamic mechanical allodynia and paradoxical heat sensation. Patients were examined on the dorsum of the right foot, and the vibration detection threshold was measured on the right medial malleolus. Equista (Germany) a QST data analysis system that transfers data into standard normal distribution (*z*-scores), adjusting for age, sex and body localization was used [25].

The predefined case definition of DPN was as proposed by the Toronto Diabetic Neuropathy Expert Group [8]. In the definition, there are three levels of certainty: possible DPN, which requires at least one of either sensory symptoms, signs or reduced ankle reflexes; probable DPN, which requires two of the three; and definite DPN, which requires one of the three plus abnormal NCS or IENFD. Clinical diagnosis of painful DPN was made in accordance with the NeuPSIG grading system and adapted to painful DPN [7]. Possible painful DPN was defined as pain in both feet/legs and a diagnosis of diabetes, probable painful DPN as pain in both feet/legs and sensory signs in the feet/legs, and definite painful DPN as pain in both feet/legs and sensory signs in the feet/legs plus an abnormal IENFD or NCS (Fig. S1). The symptoms and signs of neuropathy and pain reported in the DN4, MNSI, TCNS and UENS were not used to diagnose DPN and painful DPN.

The study was approved by the Regional Research Ethics Committee of Central Denmark Region (file number 1-10-72-130-16). The Danish National Committee on Health Research Ethics (file number S-20100082) approved the DD2 project. The Danish Data Protection Agency (file number 2008-58-0035) approved the DD2 project, and the study was registered at Aarhus University, internal notification number 62908-250. All patients and controls gave written informed consent.

Statistics

For the prevalence calculations the patients were divided into two strata based on the MNSIq and DN4q in the questionnaire study (MNSIq ≥ 4 and/or DN4q ≥ 3 and those without), and by weighting the prevalences by the strata sizes the prevalence of definite DPN and painful DPN were estimated with a 95% confidence interval (CI) [26]. An example of this calculation is described in Fig. S2.

Assuming that clinical and demographic characteristics were monotonically increasing or decreasing when going from no DPN to definite DPN, the hypothesis of no relationship was tested using Spearman's rank-order correlation. The correlation analyses were also stratified by sex.

The sensitivity and specificity of the questionnaires (MNSIq and DN4q) and screening instruments (MNSI, TCNS, UENS) were calculated exclusively for the random sample of 165 patients as those calculations depend on patient sampling. Positive predictive values (PPVs) and negative predictive values (NPVs) and the receiver operating characteristic area were calculated for the whole cohort of the examined patients, as they are independent of sampling. The gold standard (the comparator) was definite DPN and painful DPN. The same calculations were performed for at least probable (definite and probable) DPN and painful DPN as a gold standard. All results were presented with 95% exact binomial confidence intervals. McNemar's test for paired data was used to compare the prevalence of MNSIq ≥ 4 between the questionnaire survey study and the clinical study. McNemar's test was also used to compare proportions of patients with abnormal QST and IENFD (dichotomous), two different measures for small nerve fibers.

Study data were collected and managed using Research Electronic Data Capture (REDCap) hosted at Aarhus University. Double data entry was performed. For data analysis and figures, STATA (version 14; StataCorp LP, College Station, TX, USA) and R Core Team (version 3.6.1, 2019, Vienna, Austria) were used.

Results

Out of 5514 eligible patients in the DD2 cohort who completed the questionnaire [15], 1461 (26.5%) were invited for a clinical examination (Fig. 1). Of these, 389 (26.6%) patients agreed to participate (Fig. 1). There were no differences between responders and non-responders with respect to either demographic characteristics or symptoms of DPN and painful DPN reported in the questionnaire survey study (Table S2). The time from participation in the questionnaire survey to the clinical study was a median of 1.29 (interquartile range 0.82; 1.71) years.

Of the 389 patients, 126 had definite DPN, of whom 53 had painful DPN. A further 88 had probable, 53 possible and 10 subclinical DPN, where NCS or skin biopsy results were abnormal in the absence of symptoms or signs (Fig. 2). In addition, 49 had polyneuropathy of other causes or other neurological or pain disorders indistinguishable from the symptoms of DPN and painful DPN (Fig. 2), whilst 63 had no DPN.

Based on a clinical diagnosis of definite DPN and painful DPN in the cohort of 389 patients, the

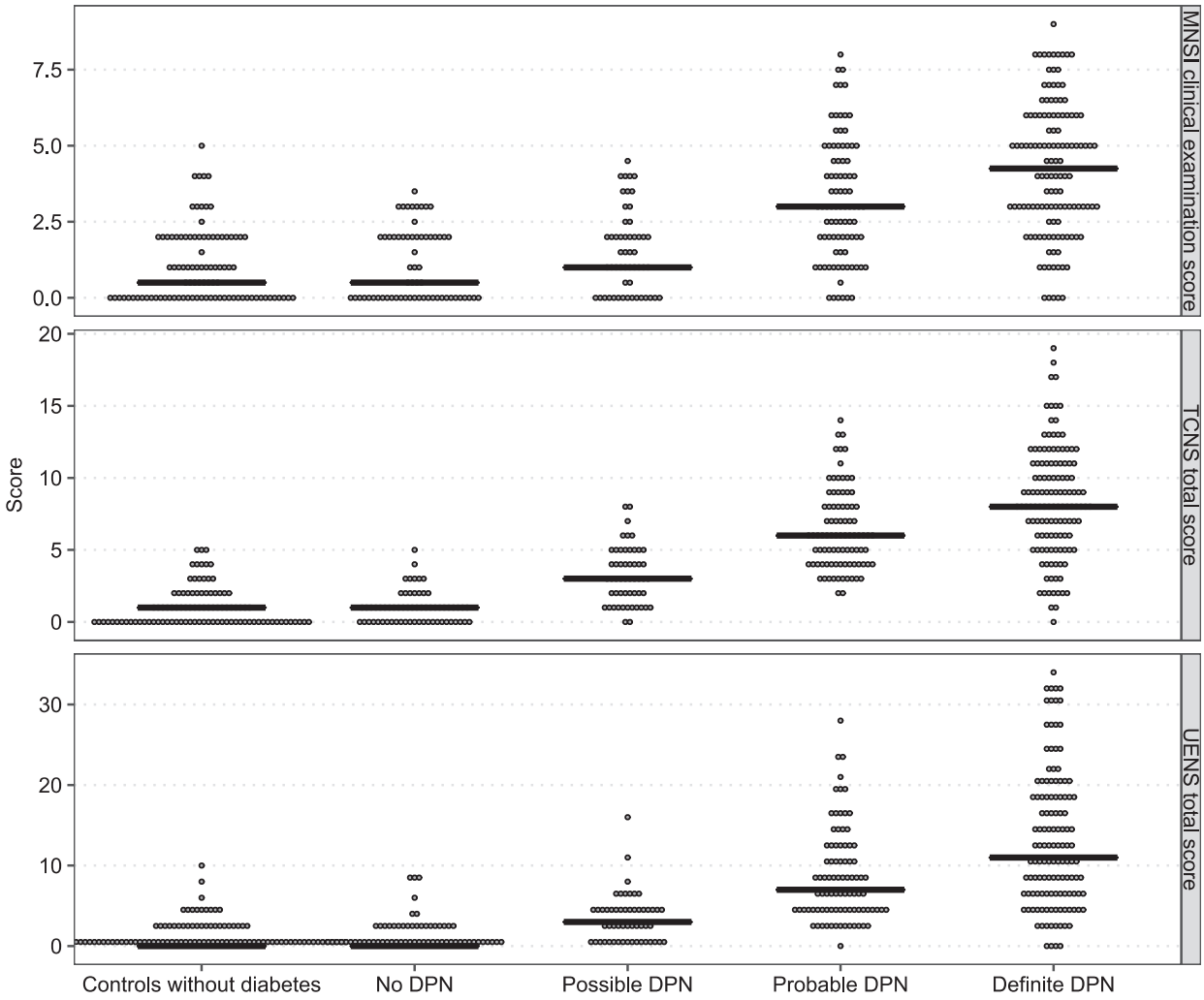

Figure 2 The median TCNS, UENS and MNSI examination scores and the correlation between the DPN groups inclusive controls without diabetes and the scores. MNSI r_s 0.61, $P < 0.001$; TCNS r_s 0.79, $P < 0.001$; UENS r_s 0.73, $P < 0.001$. TCNS, Toronto Clinical Neuropathy Scoring System: 0–5 no neuropathy, 6–8 mild neuropathy, 9–11 moderate neuropathy, >11 severe neuropathy (highest possible score is 19). UENS, the Utah Early Neuropathy Scale (highest possible score is 42). MNSI, Michigan Neuropathy Screening Instrument, examination part (highest possible score is 10). r_s Spearman's rho. DPN, diabetic polyneuropathy.

estimated prevalence of definite DPN and painful DPN in the questionnaire survey cohort of 5514 patients, with a median time from diabetes diagnosis of 5.9 years (interquartile range 4.2; 7.1), was 22.7% (95% CI 17.5; 28.0) and 5.4% (95% CI 3.5; 7.3) respectively. For probable and definite DPN the prevalence was 43.9% (95% CI 37.3; 50.5) and for possible, probable and definite DPN it was 62.2% (95% CI 55.5; 68.8). For painful DPN the prevalence of at least probable painful DPN was 11.5% (95% CI 8.2; 14.9) and at least possible painful DPN 12.0% (95% CI 8.6; 15.3).

The sensitivity of an MNSIq score ≥ 4 to detect definite DPN was 25.7% (95% CI 12.5; 43.3) and the specificity was 84.6% (95% CI 77.2; 90.3) (Table 1). The sensitivity of the DN4q score of ≥ 3 together with pain in both feet in detecting definite painful DPN was 80% (95% CI 44.4; 97.5) and the specificity was 89.9% (95% CI 83.6; 94.3) (Table 1). Values for PPV, NPV, the TCNS, MNSI examination and the UENS are provided in Table 1. There was a positive correlation between clinical scores and increased certainty of the DPN diagnosis from controls without diabetes to definite DPN for the MNSI clinical examination, TCNS and UENS. For controls without diabetes, and patients with no DPN and possible DPN, the median scores were similar (Fig. 2).

Comparing the MNSIq answers from the questionnaire survey study with answers from the day of the clinical examination in the cohort of 389 patients,

Table 1 Diagnostic accuracy of questionnaires and clinical scoring instruments with different cutoff points to detect definite DPN and the DN4q and pain in both feet to detect definite painful DPN

	MNSIq ≥ 3*	MNSIq ≥ 4*	MNSIq ≥ 5*	DN4 ≥ 3 and pain in both feet**	TCNS > 5*	UENS > 3*	MNSI clinical* examination > 2
Definite DPN*							
Definite painful DPN**							
Sensitivity	37.1 (21.5; 55.1)	25.7 (12.5; 43.3)	25.7 (12.5; 43.3)	80.0 (44.4; 97.5)	62.9 (44.9; 78.5)	82.9 (66.4; 93.4)	74.3 (56.7; 87.5)
Specificity	73.1 (64.6; 80.5)	84.6 (77.2; 90.3)	88.5 (81.7; 93.4)	89.9 (83.6; 94.3)	74.6 (66.2; 81.8)	50.0 (41.1; 58.9)	68.5 (59.7; 76.3)
Correctly classified	65.5	72.1	75.2	89.2	72.1	57.0	69.7
ROC area	0.55 (0.46; 0.64)	0.55 (0.47; 0.63)	0.57 (0.49; 0.65)	0.85 (0.72; 0.98)	0.69 (0.60; 0.78)	0.66 (0.59; 0.74)	0.71 (0.63; 0.80)
Positive predictive value	41.0 (34.2; 47.9)	42.9 (35.1; 51.1)	46.2 (37.0; 55.6)	43.3 (33.6; 53.3)	52.7 (45.2; 60.2)	44.7 (38.5; 51.0)	48.0 (41.0; 55.1)
Negative predictive value	77.7 (70.8; 83.5)	74.7 (68.6; 80.1)	73.7 (68.0; 78.9)	97.0 (94.2; 98.7)	85.5 (80.0; 90.0)	91.0 (84.9; 95.3)	84.9 (78.9; 89.7)
At least probable DPN*							
At least probable painful DPN**							
Sensitivity	40.5 (29.3; 52.6)	27.0 (17.4; 38.6)	24.3 (15.1; 35.7)	72.7 (49.8; 89.3)	–	–	–
Specificity	80.2 (70.6; 87.8)	90.1 (82.1; 95.4)	93.4 (86.2; 97.5)	95.2 (89.9; 98.2)	–	–	–
Correctly classified	62.4	61.8	62.4	91.9	–	–	–
ROC area	0.60 (0.53; 0.67)	0.59 (0.53; 0.65)	0.59 (0.53; 0.64)	0.84 (0.74; 0.94)	–	–	–
Positive predictive value	66.7 (59.9; 73.0)	67.9 (60.0; 75.2)	73.1 (64.2; 80.8)	69.2 (59.4; 77.9)	–	–	–
Negative predictive value	58.7 (51.1; 66.0)	53.6 (47.0; 60.2)	53.0 (46.8; 59.0)	93.3 (89.6; 96.0)	–	–	–

Data are percent with 95% confidence interval. The questionnaires (MNSIq and DN4q) were filled in on the day of the clinical examination. The MNSIq consists of 13 questions and the DN4q of seven. There were 17 with missing data on the DN4q. The sensitivity and specificity were calculated in the sample of randomly selected patients (165) and positive and negative predictive values for all 389 patients. Gold standard (the comparator) was patients with definite DPN/painful DPN or at least probable DPN/painful DPN compared to those without definite DPN/painful DPN or without at least probable DPN/painful DPN. MNSIq, Michigan Neuropathy Screening Instrument questionnaire; DN4q, Douleur Neuropathique 4 questionnaire; TCNS, Toronto Clinical Neuropathy Scoring System; UENS, Utah Early Neuropathy Score; ROC area, receiver operating characteristic area (sensitivity + specificity)/2; –, not applicable.

Table 2 Patient characteristics of the 330 diabetes patients with no DPN, possible DPN, probable DPN and definite DPN and the correlation between characteristics and the DPN groups

	No DPN	Possible DPN	Probable DPN	Definite DPN	r_s/P
N	63	53	88	126	
Female sex, *n* (%)	31 (49.2)	28 (52.8)	37 (42.1)	41 (32.5)	−0.15/0.006
Age (years)	64.1 (58.3; 70.7)	64.6 (57.3; 70.9)	67.7 (59.1; 72.4)	67.2 (58.0; 72.3)	0.10/0.064
Time since diabetes diagnosis (years)	5.5 (4.2; 6.7)	5.9 (4.2; 7.1)	5.8 (4.0; 7.0)	6.1 (4.5; 7.4)	0.08/0.14
BMI (kg/m^2)	30.0 (27.5; 34.3)	29.4 (26.7; 35.9)	29.8 (26.5; 33.5)	32.8 (29.2; 37.6)	0.19/<0.001
Height (cm)	170.0 (162.0; 178.0)	169.0 (163.0; 177.0)	174.5 (165.0; 180.0)	177.0 (168.8; 181.6)	0.23/<0.001
Weight (kg)	91.8 (75.7; 101.0)	88.0 (76.5; 102.6)	88.3 (77.2; 102.5)	105.5 (86.9; 114.8)	0.28/<0.001
Waist circumference (cm)	104.8 (95.4; 113.1)	104.0 (96.8; 114.3)	104.3 (94.6; 114.9)	113.3 (104.3; 123.8)	0.28/<0.001
Systolic blood pressure (mmHg)[a]	138.0 (126.0; 145.0)	135.0 (129.0; 144.5)	136.0 (123.0; 148.0)	143.0 (131.0; 151.3)	0.14/0.010
Diastolic blood pressure (mmHg)[a]	82.0 (75.0; 91.0)	82.0 (75.5; 90.0)	82.0 (74.3; 89.0)	83.0 (78.0; 89.0)	0.02/0.66
HbA_{1c} (mmol/mol)	49.0 (45.0; 53.0)	49.0 (45.0; 53.0)	49.0 (43.5; 57.0)	50.0 (45.0; 58.0)	0.09/0.10
Total cholesterol (mmol/l)	4.2 (3.7; 4.6)	4.1 (3.6; 4.8)	4.1 (3.5; 4.7)	3.9 (3.3; 4.5)	−0.07/0.22
Triglycerides (mmol/l)	1.8 (1.3; 2.7)	2.0 (1.4; 2.6)	1.6 (1.3; 2.4)	2.0 (1.5; 2.9)	0.08/0.16
Alcohol (>7/14 units/week female/male)[b]	5 (7.9)	7 (13.2)	9 (10.2)	14 (11.1)	0.02/0.71
Current smoker	9 (14.3)	9 (17.0)	16 (18.4)	17 (13.6)	−0.02/0.73
Physical activity (≥3 times/week)[c]	20 (40.0)	14 (30.4)	39 (47.6)	41 (34.2)	−0.03/0.60
Treatment with					
Antidiabetics other than insulin[d]	51 (81.0)	48 (90.6)	81 (92.1)	109 (86.5)	0.03/0.61
Insulin or insulin analogs	7 (11.1)	4 (7.6)	5 (5.7)	32 (25.4)	0.19/<0.001
No other than dietary	10 (15.9)	3 (5.7)	7 (8.0)	13 (10.3)	−0.03/0.59
Antihypertensives	42 (66.7)	37 (69.8)	56 (63.6)	100 (79.4)	0.11/0.043
Cholesterol lowering drugs	48 (76.2)	45 (84.9)	69 (78.4)	102 (81.0)	0.02/0.70
Anticoagulants[e]	28 (44.4)	24 (45.3)	39 (44.3)	64 (50.8)	0.05/0.34
Analgesics[f]	16 (25.4)	16 (30.2)	31 (35.2)	57 (45.2)	0.16/0.004
Asymptomatic DPN	–	33 (62.3)	13 (14.8)	28 (22.2)	–

Data are shown as median (interquartile range) or *n* (%). Clinical characteristics are provided for the 330 patients with either possible, probable or definite DPN, not including subclinical DPN and other causes of neuropathy, neuropathy symptoms or pain. r_s Spearman´s rho; –, not applicable; BMI, body mass index; HbA_{1c}, glycated hemoglobin; NSAIDs, nonsteroidal anti-inflammatory drugs; SNRIs, serotonin-noradrenaline reuptake inhibitors; TCA, tricyclic antidepressants; missing data, <0.01%, except for physical activity with 9.7% (32) missing. [a]The recommended goal for hypertension treatment in patients with diabetes in Denmark is a systolic blood pressure of 120–130 mmHg and a diastolic blood pressure of 70–80 mmHg. [b]The maximum amount of alcohol units per week recommended by the Danish Health Authority. [c]On average, how often are you physically active per week. [d]Antidiabetics other than insulin: metformin, dapagliflozin, liraglutide, sitagliptin, glimepiride, glibenclamide, empagliflozin. [e]Anticoagulants: acetylsalicylic acid, clopidogrel, warfarin and novel oral anticoagulants. [f]Analgesics: paracetamol, NSAIDs, TCAs, gabapentin, pregabalin, SNRIs, tramadol, codeine and morphine.

there was a change in mean sum scores of −0.31 (SD 1.7), yet 37.2% changed status from MNSIq ≥ 4 to MNSIq < 4 and 14.3% the other way around ($P < 0.001$) (Table S3).

In general, increasing certainty of the DPN diagnosis (from no DPN to definite DPN) was weakly correlated to different patient characteristics. The proportion of males increased from no DPN to definite DPN. Patients with definite DPN had higher body mass index, weight, waist circumference, height and systolic blood pressure compared to those with no DPN, which persisted for body mass index, weight and waist circumference after stratifying for sex (Tables 2, S4 and S5). Patients with definite DPN used more insulin or insulin analogs, antihypertensive drugs and analgesic drugs compared to the other groups, but the same was not true for other antidiabetics than insulin or insulin analogs or dietary treatment (Table 2).

In the cohort of 389 patients, 386 (99.2%) had results for IENFD and/or NCS. Of these, 31.4% had abnormal IENFD and 23.9% abnormal NCS (Table S6). Of the 126 patients with definite DPN, 113 had results for both IENFD and NCS. Of these, 19 (16.8%) had abnormal NCS alone, 47 (41.6%) had abnormal IENFD alone and 47 (41.6%) had both (data not shown). The agreement between two different small fiber measures, IENFD and cold and warm detection thresholds on the QST, was poor. Of those with abnormal IENFD, 35.5% had abnormal cold and/or warm detection thresholds and, vice versa, 47.8% of patients with abnormal cold and/or warm detection thresholds had abnormal IENFD, $P = 0.007$ (Table S7).

Compared to normative material [25] most patients had loss of both small and large nerve fiber function on the QST where patients with probable and definite DPN had the most pronounced sensory loss (Fig. S3 and Table S8).

Discussion

In this study, a detailed examination of patients from a large nationwide population of Danish patients with recently diagnosed type 2 diabetes was carried out. The prevalence was estimated using the gold standard definition of DPN and painful DPN and commonly used screening tools were assesed. It was found that screening tools that included a clinical examination performed better in the diagnosis of DPN compared with questionnaires alone.

Our prevalence estimates showed that there was a decrease in the prevalence with higher certainty of the DPN diagnosis, from 62.2% with at least possible DPN, 43.9% with at least probable DPN and 22.7% with definite DPN. For definite painful DPN the prevalence was 5.4%. In comparison, the prevalence of possible DPN defined as MNSIq ≥ 4 was 18% from the questionnaire survey study. The discrepancy between the prevalence of possible DPN using either the MNSIq or clinical evaluation may reflect the difference between a symptom-based questionnaire and clinical interview and examination. Interestingly, around 20% of the patients had probable DPN with either symptoms and/or signs of DPN, but with normal NCS and IENFD, possibly indicating early signs of nerve damage or neuropathy. For this large group, there is a potential for prevention and counseling or intervention such as foot care, fall prevention and change of lifestyle factors including weight loss [27,28]. Our prevalence estimates of definite DPN (22.7%) and painful DPN (5.4%) were similar to previous estimates although the prevalence of painful DPN was lower than earlier estimates [29,30]. The low prevalence could be explained by the good metabolic control in the group, short duration of diabetes and that patients with other causes of pain in the feet were carefully excluded.

In this study, the Toronto classification for the definition of possible, probable and definite DPN was used. This hierarchical system is reflected in an increase of median scores in the TCNS, UENS and MNSI clinical examination when going from possible to probable and to definite DPN. In QST parameters, patients with probable and definite DPN presented with a similar degree of sensory loss of both small and large fiber measures. Taken together, these findings indicate that, whilst a hierarchical system in classifying neuropathy is useful, there is also a substantial overlap between these different groups.

To our knowledge, the performance of the MNSIq in detecting definite DPN has not been evaluated in a cohort of solely type 2 diabetes patients or in patients with recently diagnosed diabetes. A sensitivity of the MNSIq ≥ 4 to detect definite DPN of 25.7%, a specificity of 84.6%, a PPV of 42.9 and an NPV of 74.7% were found. Herman and colleagues [11] found a sensitivity of 40% and a specificity of 92%, a PPV of 69% and an NPV of 78% for MNSIq ≥ 4 for DPN in young (mean age 47 years) type 1 diabetes patients without severe complications or concurrent diseases. The duration and type of diabetes could account for some of the differences. The performance of the MNSI examination part, the TCNS and the UENS was worse than previously reported in patients with long-standing type 1 diabetes for the MNSI and in patients with impaired glucose tolerance and early neuropathy for the TCNS and UENS [12,31], but they performed better than the MNSIq, although the MNSIq had the highest specificity. It is possible that better performances would have been obtained if other criteria for DPN had been used. However, there is currently no evidence from this or other studies indicating better gold standards than the present to classify patients with painful or non-painful DPN. The combination of the presence of pain in both feet and a positive DN4q score performed well, supporting the use of these screening questionnaires for the detection of painful DPN.

Small fiber involvement was assessed using structural measures with IENFD and functional measures with determination of cold, warm detection thresholds and thermal sensory limen. The overall agreement between the functional and structural measures was weak, which is not surprising. In a previous meta-analysis it was shown that there is in general weak correspondence between structural and functional measures [32]. The reason for this lack of relationship, or at best weak relationship, between small fiber function and small nerve fiber count penetrating the skin from dermis into epidermis is not known. One reason might be that in DPN there is both degeneration and regeneration of nerve fibers [1] and it is currently unknown to what extent regenerating fibers may contribute to both the structural and functional measures [32].

There was low consistency in the MNSIq answers from the time of the questionnaire survey study to the clinical study. This may be explained by fluctuations in DPN symptoms, the time difference between the two assessments, and the low sensitivity of the MNSIq, and suggests that questionnaires should not stand alone in the screening and diagnosis of DPN.

In this study, widely accepted gold standards for the diagnosis of definite DPN and definite painful DPN were used. Data were not collected on other diabetes complications than DPN; these data are described in detail for the DD2 cohort elsewhere [33,34]. A structured neurological examination was

performed and a detailed history was obtained in order to exclude other causes of polyneuropathy; however, the findings were not verified with, for example, B12 vitamin deficiency by blood samples. Even though the diagnosis of DPN was based on the bedside neurological examination together with history and NCS/IENFD, incorporation bias cannot be ruled out, as some of the measures (i.e. reflexes and vibration measures) were also included in the scoring of the clinical neuropathy scales. Stratified sampling, based on questionnaire responses, was possible because patients were included from a known, representative population of 5514 type 2 diabetes patients. Therefore, our sample size of 389 combined with a thorough examination provided strong estimates of the prevalence with relatively narrow confidence intervals. Lastly, with 389 responders from a total of 1461 invited, a selection bias is possible although there were no differences in clinical characteristics between the groups (Table S2) and the questionnaire study cohort from which was sampled was representative of recently diagnosed Danish type 2 diabetes patients [15,33].

In conclusion, DPN in patients with recently diagnosed diabetes is common, with one in five patients having confirmed DPN and almost half having at least symptoms and/or signs of DPN. However, painful DPN was not as common as previously reported. Neuropathy scales including both questions and clinical examination, or clinical examination alone, were more accurate in the detection of definite DPN than questionnaires alone. Therefore, it is better to use the gold standard even in epidemiological studies if possible.

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Disclosure of conflicts of interest

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Data availability statement

Data available on request from the authors.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the 97 controls without diabetes.

Table S2. Clinical characteristics of non-responders versus responders of those invited to participate based on data from the questionnaire survey.

Table S3. Change in MNSIq scores from the questionnaire survey to the clinical study for all 389 patients who participated in the clinical study.

Table S4. Clinical characteristics and neuropathy groups divided by sex.

Table S5. Correlation between DPN groups and clinical characteristics divided by sex.

Table S6. Small and large nerve fiber involvement measured by nerve conduction studies and intraepidermal nerve fiber density for all 389 patients including number of patients with missing data.

Table S7. Agreement between two small fiber measures, quantitative sensory testing and intraepidermal nerve fiber density, for the 333 with data on both.

Table S8. Quantitative sensory testing, median (interquartile range) of *z*-scores for thermal and mechanical parameters.

Figure S1. The definition of DPN according to the Toronto classification and painful DPN adapted from the NeuPSIG grading system.

Figure S2. An example of the prevalence calculation for definite DPN and definite painful DPN in the questionnaire cohort of 5514 patients by weighting the prevalences by strata sizes.

Figure S3. Percentages of patients with values below (–%) and above (+%) the 95% CI of the German Research Network for Neuropathic Pain reference database for no DPN, possible DPN, probable DPN and definite DPN.

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